FILE 'AGRICOLA, CAPLUS, USPATFULL, BABS, CBNB, CEN, CIN, DKILIT, IFIPAT, JICST-EPLUS, PASCAL, PLASNEWS, PROMT, RAPRA, SCISEARCH, TEXTILETECH, USPAT2, WPIDS, WTEXTILES' ENTERED AT 12:00:40 ON 05 AUG 2002

L1 34451 S TUBER
L2 3954 S L1 AND STARCH
L3 30 S L2 AND AMYLOPECTIN CONTENT
L4 0 S L3 AND PURIF?(W)AMYLOPECTIN
L5 30 S L3 AND AMYLOPECTIN CONTENT
L6 18 S L5 AND 95%
L7 1 S L6 AND 95%(W)AMYLOPECTIN\

ANSWER 8 OF 30 USPATFULL
AN 2001:75158 USPATFULL
TI Process for the production of cyclodextrin
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DT Utility
FS Granted
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ICM: C12P019-16
ICS: C12P019-00; C12P019-44
EXF 435/72; 435/74; 435/98; 260/536
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is the use of amylopectin potato **starch** obtained from potatoes whose amylose formation is inhibited through breeding, or through genetic engineering or other molecular biological processes, as starting material for a process for production of cyclodextrin from potato **starch** by reaction with cyclodextrin glycosyltransferase. This **starch** starting material combines the positive effects of natural amylopectin **starch** with those of potato **starch** and is distinguished, among other properties, through low lipid and protein content and therefore higher purity. The yield of cyclodextrins. . .
SUMM The subject of this invention is a process for the production of cyclodextrin from amylopectin potato **starch** by reaction with cyclodextrin glycosyltransferase (CGTase, also known as cyclodextrin transglycosylase, EC No. 2.4.1.19).
SUMM . . . number of anhydro-glucose units in the ring which is formed. Beta-cyclodextrin is the thermodynamically favored product in the conversion of **starch** to cyclodextrin by means of CGTase. The higher-ring cyclodextrins are preferred for technical applications.
SUMM Potato **starch**: the potato can be grown with high yields per hectare even in unfavorable locations. It has low protein and lipid contents and therefore affords a very pure **starch**.
SUMM Maize and waxy maize **starch**: maize requires a warmer climate. Waxy maize ripens in high proportions. It must be grown in favorable locations with adequate. . .
SUMM A considerable disadvantage for **starch** from maize and waxy maize is the high protein and lipid content (necessitating complicated and costly purification of the **starch**).
SUMM Wheat **starch**: is a poorer substrate than potato or maize **starch** since the yield of cyclodextrin that can be produced from it is much lower.
SUMM The usual natural starches are a mixture of the two forms of **starch**, amylopectin and amylose. Amylose and amylopectin are not single substances but mixtures of polymers with different molecular weights and different. . .
SUMM . . . of the type of plant they have been obtained from. Only maize varieties of the so-called waxy type provide a **starch** which consists almost exclusively of amylopectin. In rare cases a **starch** rich in amylopectin can also be obtained from so-called waxy rice or waxy barley.
SUMM . . . ever used except on a laboratory scale. Furthermore,

fractionation of natural starches leads to uncontrolled degradation and damage to the **starch** fractions with impairment of the properties of the final products.

SUMM For this reason amylopectin **starch** is hardly ever used for technical purposes. The only use in practice involves a certain amount of waxy maize **starch** in the food industry because this generates a more pleasant feeling in the mouth than does usual **starch**.

SUMM The production of cyclodextrin from **starch** is the subject of many publications in the literature. Thus U.S. Pat. No. 3,425,910 describes a process for the production of cyclodextrin from a **starch** hydrolysate. The use of potato **starch** as **starch** starting material is mentioned. At the time of filing of the above US patent (1966) potato **starch** is a usual **starch** with an amylose content of approximately 20% by weight.

SUMM In PCT application WO 93/10255 the production of cyclodextrin from a **starch** containing at least 90% amylopectin is described whereby it is stated that the cyclodextrin obtained gives a clear solution when dissolved in water. Preferred **starch** starting materials contain 95% amylopectin or more, preferably around 99%. Waxy maize **starch**, waxy rice **starch** and waxy barley **starch** are expressly stated to be the starting materials with waxy maize **starch** being preferred. Potato **starch** and maize **starch** with normal amylose content are named in Example 1 as comparison starches for demonstrating the positive effects of using waxy maize **starch**.

SUMM . . . treated with debranching enzymes such as pullulanase or isoamylase before the addition of CGTase the level of conversion of the **starch** into cyclodextrin is increased by several percent.

SUMM . . . substrate than amylose for the production of cyclodextrin because the reaction with CGTase begins at the non-reducing end of the **starch** molecule. Since amylopectin has considerably more non-reducing ends than amylose, the level of conversion is higher when amylopectin is used. It is therefore recommended that potato **starch** be used instead of maize **starch** because potato **starch** has an intrinsically higher **amylopectin content** than maize **starch** (approximately 79% for potato compared with approximately 72% for maize).

SUMM . . . oxytoca. It did in fact prove possible to detect small amounts of cyclodextrin in the potato tubers. Extraction of the **tuber** tissue was performed using a C18 Sep-pak column which binds the cyclodextrin but not the **starch**.

SUMM U.S. Pat. No. 4 477 568 mentions among other things the use of fractionated amylopectin **starch** from a wide variety of crops, e.g. maize, wheat, sorghum, potato, tapioca, sago and rice, for the production of cyclodextrin.

SUMM However, since the **starch** fractionation processes have not been generally accepted for the above reasons, the search is still going on for a cyclodextrin. . .

SUMM . . . foregoing and other objects in view there is provided, in accordance with the invention, a process in which amylopectin potato **starch** obtained from potatoes with amylose formation inhibited as a result of breeding or of molecular biological/genetic engineering procedures is used as the starting material in a process for the production of cyclodextrin from amylopectin potato **starch** by reaction with cyclodextrin glycosyltransferase.

SUMM Recent years have seen the successful development of genetic modification of potatoes with the aim of producing **starch** which is practically free of amylose. The amylopectin potato **starch** obtained from such potatoes combines the advantages of an almost pure amylopectin possessing the original properties of the natural product, with the advantages of potato **starch**, namely

its low lipid and protein content.

SUMM Also in accordance with this invention, the amylopectin potato **starch** is best obtained from potatoes in which amylose formation is inhibited by such molecular biological/genetic engineering procedures as anti-sense technique. . .

SUMM The amylose-inhibited potato varieties used as producers of amylopectin **starch** starting material for the process of the invention provide an amylopectin **starch** with an **amylopectin content** of above 90% by weight, preferably above 95%. For the process according to the invention an amylopectin potato **starch** with an **amylopectin content** of above 98% is especially preferred.

SUMM Determination of the amylose content and the **amylopectin content** of a **starch** is carried out according to: J. H. M. Hovenkamp-Hermelink, J. N. DeVries, F. Adamse, E. Jacobsen, W. Witholt and W. J. Feenstra, "Rapid estimation of the amylose amylopectin ratio in small amounts of **tuber** and leaf tissue of the potato", Potato Res., (1988), 241-246.

SUMM The amylopectin potato **starch** according to the invention can be used as obtained from potatoes, untreated, or pretreated mechanically, thermally, chemically and/or enzymatically. Such pretreatment serves to liquefy or improve the solubility of the **starch**.

SUMM Mechanical pretreatment involves liquefying the amylopectin potato **starch** by high-speed stirring.

SUMM The **starch** can also be treated thermally at temperatures up to approximately 155.degree. C.

SUMM On the other hand, the **starch** can also be pretreated with oxidizing agents such as sodium hypochlorite.

SUMM If an amylopectin potato **starch** is treated with alpha-amylase an enzymatic degradation takes place which also renders the **starch** easier to dissolve.

SUMM Chemical pretreatment for the production of **starch** ethers, esters and/or cross-linked **starch** products is also used to advantage.

SUMM The **starch** suspension is pretreated at 100.degree. C.; the cyclization reaction then proceeds at 25.degree. C.

SUMM . . . using

Substrate used	Yield of CD (%)	Yield using pullulanase (%)	pullulanase and complexing agent (%)
Fract. maize AP	22.6	36.1	89.8
Maize **starch**	14		87.2
Waxy maize **starch**	18.6		90.6
Potato **starch**	18.9		85.9
Potato AP from transgenic potato	25.1	38.3	92.3
Wheat **starch**	15.8		86.9

SUMM . . . be limited by any theory, the following may explain why the highest yields of cyclodextrins are obtained from amylose-free potato **starch**:

SUMM The small fragment fraction is lowest for the potato **starch** (M. T. Kalichevsky, P. D. Orford and S. G. Ring, "The retrogradation and gelation of amylopectins from various botanical sources",. . .

SUMM . . . cyclodextrins obtained using amylopectin starches from transgenic potatoes are higher than the yields obtained from reaction mixtures with waxy maize **starch** (J. W. Shieh and A. Hedges, PCT application WO 93/10255 (1993)). A possible explanation is the higher content of Fraction. . .

SUMM In accordance with an additional feature of the invention, it has been found particularly advantageous to use an amylopectin potato **starch** with a degree of polymerization level (DP) of .gtoreq.50.

As can be seen in Table 3 below, the yield of cyclodextrin increases with increasing DP of the **starch** used as starting material.

SUMM The high purity of the potato **starch** (low fat and protein content) is an advantage in isolation of the cyclodextrins from the reaction mixture, as manifested for. . .

SUMM TABLE 4

	% in dry substance	
Starch	Protein	Lipid
Maize	0.2-0.4	0.5-0.9
Potato	0.05-0.1	0-0.1

DETD 100 g of amylose-free potato **starch** from transgenic potato was suspended in 1 litre of water and gelatinized by heating to 100.degree. C. within 30 minutes.. . . of 0.1 in a solution prepared as follows: After incubation of a mixture of 0.5 ml of 1% soluble rice **starch**, 0.1 mol of 0.5 M acetate buffer [pH 3.6] and 0.1 ml of enzyme solution at 40.degree. C. for 1h. . .

CLM What is claimed is:

1. A process for the production of cyclodextrin from amylopectin potato **starch** in which amylopectin potato **starch** containing at least 90% amylopectin and obtained from potato having amylose formation inhibited, as a result of breeding or of. . . from the reaction mixture in enhanced yield and purity compared to an otherwise comparable process with fractionated amylopectin of maize **starch** as substrate.

2. The process according to claim 1, in which amylopectin potato **starch** is obtained from potatoes whose amylose formation is inhibited through use of anti-sense technique.

3. The process according to claim 1, in which amylopectin potato **starch** is obtained from potatoes whose amylose formation is inhibited through use of cosuppression.

4. The process according to claim 1, in which amylopectin potato **starch** with an **amylopectin content** of at least 95% is used.

5. The process according to claim 4, in which amylopectin potato **starch** with an **amylopectin content** of at least 98% is used.

6. The process according to claim 1, in which mechanically and/or thermally and/or chemically and/or enzymatically pretreated amylopectin potato **starch** is used.

7. The process according to claim 6, in which amylopectin potato **starch** is mechanically pretreated by high-speed stirring.

8. The process according to claim 6, in which amylopectin potato **starch** is thermally pretreated at temperatures up to approximately 155.degree. C.

9. The process according to claim 6, in which amylopectin potato **starch** is chemically pretreated with acid.

11. The process according to claim 6, in which amylopectin potato **starch** is chemically pretreated with an oxidizing agent.

13. The process according to claim 6, in which amylopectin potato **starch** is enzymatically pretreated with alpha-amylase.

14. The process according to claim 6, in which amylopectin potato

starch is chemically pretreated by etherifcation, esterification and/or cross-linking.

15. The process according to claim 6, in which amylopectin potato **starch** is enzymatically pretreated with a debranching enzyme.

17. The process according to claim 1, in which amylopectin potato **starch** has a DP.gtoreq.50.

18. The process according to claim 1, in which the conversion of the **starch** with CGTase is carried out in the presence of a complexing agent for cyclodextrin.

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